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Immunohistochemical study of placental lactogen in the trophoblast in immaturity of the chorionic tree of the placenta on the background of iron deficiency anemia in pregnant women

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Abstract

The study of placental lactogen by immunohistochemistry in histological sections of the placenta during physiological pregnancy and on the background of iron deficiency anemia in the aspect of chronic placental insufficiency. It is established that during gestation against the background of iron deficiency anemia decreases the production of placental lactogen by syncytiotrophoblast of the chorionic villi and correlates with the state of the blood of the pregnant woman. The optical density of immunohistochemical staining for placental lactogen of the free trophoblast reflects the degree of maturation of the chorionic tree of the placenta in

iron deficiency anemia in pregnant women. Chronic placental insufficiency caused by immaturity of the chorionic villi can be compensated at the level of metabolism.

Key words: placental lactogen; iron deficiency anemia; trophoblast; chorionic tree immaturity.

Introduction. Iron deficiency anemia in pregnant women (IDA) is an urgent problem of modern obstetrics, its frequency ranges from 28 to 85%. Previous studies have shown that the placenta during gestation on the background of iron deficiency anemia compared with physiological pregnancy is characterized by immaturity of the chorionic tree [1]. Signs of inhibition of development of a chorionic tree of a placenta are shown by big percent of gestational less mature free structures: trunk "early", mesenchymal, embryonic, intermediate immature, trophoblastic and free processes, and also disturbance of transitions of terminal vortex specializations in special villi [2, 9].

Immaturity of the chorionic tree of the placenta according to gestational age is a morphological prerequisite for chronic placental insufficiency (CPI), which can be compensated or decompensated [3, 4]. A fundamentally important criterion for the degree of compensation of CPI is the level of production of placental hormones, among which for the last weeks of pregnancy the most important is placental lactogen (PL) [3]. We consider it appropriate to study the production of placental lactogen by placental trophoblast in gestational immaturity of the placenta on the background of IDA for further understanding of the morphogenesis of chronic placental insufficiency.

The aim of the study. To establish quantitative parameters of placental lactogen content in the trophoblast of free placental structures in iron deficiency anemia of pregnant women in the aspect of chronic placental insufficiency by immunohistochemical method.

Material and methods. Morphological studies were conducted at the Department of Pathological Anatomy of Bukovynian State Medical University (Chernivtsi, Ukraine) in compliance with the basic bioethical provisions of the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), Helsinki Declaration of the World Medical Association on ethical principles of scientific medical research with human participation (1964–2008), as well as the order of the Ministry of Health of Ukraine № 690 dated 23.09.2009.

140 placentas of observations of physiological pregnancy and gestation on the background of IDA I, II and III st. of severity without CPI and with clinical signs of CPI were studied. The term of childbirth is 37-40 weeks. IDA is a chronic pathology, so the study took

only cases of complete clinical development of the disease. The criterion for the severity of IDA on the basis of medical records was the concentration of hemoglobin in the blood of the pregnant woman: I st. of IDA corresponded to a decrease in the concentration of hemoglobin to 100-91 g / l, II st. - 90-71 g / l, III st. - below 71 g / l. In all cases, the anemia was hypochromic. According to the complex of clinical data during pregnancy, the diagnosis "Chronic placental insufficiency syndrome" was made or rejected, which was specified in the pathomorphological examination of manure. The number of observations in specific study groups are given in tables 1 and 2.

Placental material was fixed for 24 hours in a neutral buffered 10% formalin solution, followed by dehydration in an ascending battery of alcohols and paraffin at 58 °C. Histological sections with a thickness of 5 µm were used for the production of immunohistochemical techniques using primary antibodies against placental lactogen. Visualization of primary antibodies was performed with a polymer system DAKO with the dye diaminobenzidine [6]. The concentration of placental lactogen was judged on the basis of the optical density of immunohistochemical specific color, which was measured in relative units of optical density by computer microdensitometry (from 0 - no color, absolute transparency; to 1 - maximum color, absolute opaque). ImageJ software (version 1.48v, free license, W. Rasband, National Institute of Health, USA, 2015) [7], by logarithmic conversion of the average brightness in each probe. Optical color density was used as a measure of immunohistochemical concentration. For each indicator, the arithmetic mean and its error were calculated, and comparisons between study groups were performed using the two-sided odd Student test in the environment of the computer program PAST 3.06 (free license, O. Hammer, 2015). [8]. Shapiro Wilki was previously tested for normality in the samples using the same computer program. Differences at $p \leq 0.05$ were considered statistically significant.

Results of the research. A fundamentally important criterion for the diagnosis of chronic placental insufficiency is a reduced level of production of placental hormones [4]. For the last weeks of pregnancy, placental lactogen is of the greatest importance among placental hormones [5].

The results of immunohistochemical studies to determine the concentration of PL in the syncytiotrophoblast of the chorionic villi of the placenta during physiological pregnancy and against the background of IDA in terms of chronic placental insufficiency are presented in tables 1 and 2.

These data indicate that in the observations of gestation with signs of CPI without anemia significantly reduces the average concentration of PL in the syncytiotrophoblast of the

chorionic villi (0.326 ± 0.0034 AU optical density) compared with the physiological course of pregnancy (0.345 ± 0.0027 in .o. wholesale density).

Table 1 - Optical density of immunohistochemical staining for placental lactogen in the trophoblast of the chorionic villi of the placenta (control groups)

Optical color density (Acting wholesale density)	Comparison groups			
	Physiological pregnancy (n=20)	CPI without IDA (n=20)	IDA without CPI (n=38)	
			I st (n=20)	II st (n=18)
Placental lactogen	$0,345 \pm 0,0027$	$0,326 \pm 0,0034$	$0,342 \pm 0,0030$	$0,340 \pm 0,0037$

At the same time, in the groups of observations in the absence of clinical signs of CPI, no statistically significant decrease in the concentration of the hormone in these structures of the placenta in IDA I st. In placentas at IDA of the II st. concentration of hormone in a syncytiotrophoblast decreases to $0,340 \pm 0,0037$ v.o. wholesale densities. Such results of immunohistochemical researches of PL can be explained by insignificant inhibition of development of a chorion tree of placentas at IDA of the I and II st. and certain compensation of CPI at the level of a metabolism (without deficit of weight of a fruit, the newborn) in this group of supervision.

Table 2 - Optical density of immunohistochemical staining for placental lactogen in the trophoblast of the chorionic villi of the placenta (main groups)

Optical color density (Acting wholesale density)	The main groups		
	CPI with IDA I st (n=22)	CPI with IDA II st (n=24)	CPI with IDA III st (n=16)
Placental lactogen	$0,338 \pm 0,0034$	$0,329 \pm 0,0034$	$0,322 \pm 0,0031$

In all observations of pregnancy on the background of iron deficiency anemia with the presence of clinical signs of CPI, a progressive decrease in the immunohistochemical concentration of PL in the syncytiotrophoblast was observed: in IDA I st. - 0.338 ± 0.0034 acting wholesale density, II st. - $0,329 \pm 0,0034$ v.o. wholesale density, III st. - $0,322 \pm 0,0031$ v.o. wholesale densities.

Taking into account the peculiarities of the structure of the chorionic tree of the placenta in observations with IDA, syncytiotrophoblast production of placental lactogen, we

can conclude that the optical density of immunohistochemical staining for placental lactogen of the free trophoblast reflects the degree of equivalents of chronic placental insufficiency.

These patterns of concentration of placental lactogen in the syncytiotrophoblast of the chorionic villi are illustrated by digital micrographs in Figure 1.

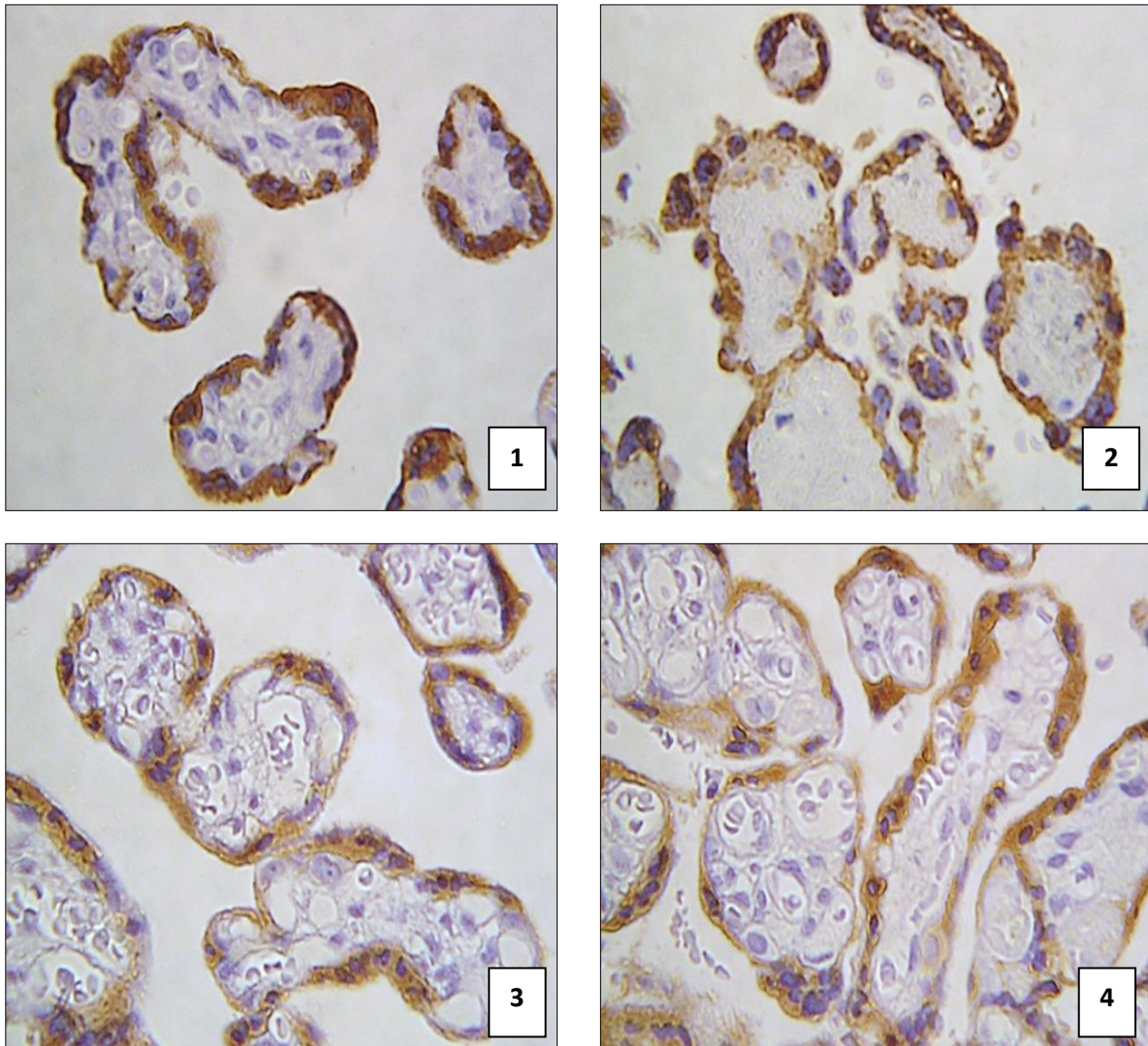


Fig. 1. The concentration of placental lactogen in the syncytiotrophoblast of the chorionic villi of the placenta with the physiological course of pregnancy and on the background of IDA in terms of chronic placental insufficiency: 1 - placenta with the physiological course of pregnancy; 2 - placenta with pregnancy on the background of IDA without clinical manifestations of chronic placental insufficiency; 3 - placenta with clinical manifestations of chronic placental insufficiency; 4 - placenta with clinical manifestations of chronic placental insufficiency on the background of IDA. Immunohistochemical procedure with primary antibodies against placental lactogen and visualization of primary antibodies by streptavidin-biotin method using diaminobenzidine. Additional staining of cell nuclei with Mayer hemalaun. Ob.40h, Ok.10 x.

Conclusions

1. During gestation on the background of iron deficiency anemia, the decrease in the production of placental lactogen by syncytiotrophoblast of the chorionic villi correlates with the severity of anemia.

2. The optical density of immunohistochemical staining for placental lactogen of the free trophoblast reflects the degree of maturation of the placental chorionic tree in iron deficiency anemia in pregnant women.

3. Chronic placental insufficiency caused by immaturity of the chorionic villi of the placenta in iron deficiency anemia in pregnant women can be compensated at the level of metabolism.

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